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# Time resolved SAXS measurement of the oligomerization of CEL-III, a hemolytic lectin from sea cucumber

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## Introduction

CEL-III is a $Ca^{2+}$-dependent, Gal/GalNAc-specific lectin purified from sea cucumber *Cucumaria echinata*, which shows haemolytic activity, especially toward human and rabbit erythrocytes [1]. Hemolysis is caused by the colloid osmotic rupture of the erythrocyte membrane due to the formation of ion-permeable pores by CEL-III oligomer after it has bound to carbohydrate receptors on the cell surface. Oligomerization of these proteins can be induced not only in lipid membranes but also in solution under the appropriate conditions. CEL-III forms an oligomer in solution when complexed with lactose at high pH values and in the presence of high concentrations of salt, e.g. at pH 10 and with 1 M NaCl [2]. Previous report shows that the molecular mass of the oligomer is determined as 1019 kDa or 25-mer from its forward scattering value by small-angle X-ray scattering (SAXS) and dissociate into hexamer in the presence of detergent [3,4]. Hence, time resolved SAXS measurements were carried out in the presence of detergent to resolve the structural change in oligomerization.

## Materials and Methods

CEL-III was purified from the body fluid of C. echinata using column chromatography on lactosyl-Cellulofine, GalNAc-Cellulofine, and Sephacryl S-200 as previously described [5]. Oligomerization was carried out under the 10 mM lactose, 1 M NaCl, 10 mM CaCl2 and 20 mM Tris-HCl buffer, at pH 7.5. SAXS measurements and analysis were described elsewhere [6].

## Results and Discussion

CEL-III monomer was purified from *C. echinata* successfully and we measured SAXS under the various pH conditions to find appropriate time scale to measurement. Measurements should be within 20 minutes to avoid radiation damage of monomeric CEL-III. The presence of carbohydrate and $Ca^{2+}$ is need to oligomerization, so we changed pH in oligomerization solution. Above the pH 8.5, oligomerization finished within 2 minutes. At the pH 7.0 and 7.5, CEL-III monomer did not associate into oligomer after 2 minutes. Thus, we measured SAXS at pH 7.5 and 8.0 for 20 minutes. Figure 1(a) shows a time course of Guinier plot under the pH 7.5. In lower Q region, the slope in the Guinier region was changed. This indicates that the oligomerization proceed under this condition.

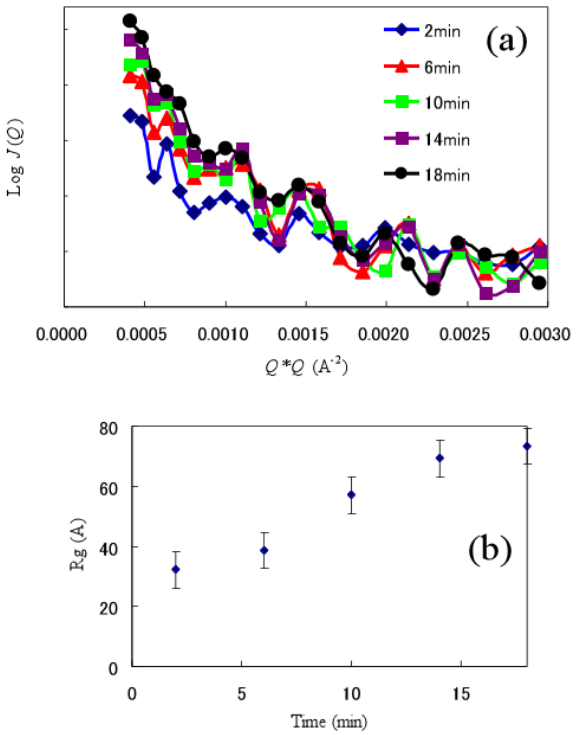


Figure 1. Time resolved SAXS measurements. Time course of Guinier plot (a) and Rg values (b).

Figure 1(b) shows a change of Rg values under the same condition. Rg value was changed from 32.1 to 73.3. The Rg value of the monomer and oligomer in the presence of detergent is 24.5 and 62.1, respectively. This result indicates that the oligomerization speed depends on the pH and we can analyze the strucutural change in oligomerization at pH 7.5 in the presence of detergent.

## References

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